# Supplementary Information Appendix for

## Structural basis for *Acinetobacter baumannii* biofilm formation

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**This PDF file includes:**

## *SI* Materials and Methods

**Bacterial strains and plasmids.** Characteristics and source of the bacterial strains used in this study are given in Supplementary Table S1. Oligonucleotides are listed in Supplementary Table S2.

The *Csu* gene cluster was amplified by PCR from chromosomal DNA of *Acinetobacter baumannii* Bouvet and Grimont (ATCC® 19606D-5™) using primers CsuABABCDE_N-F and CsuABABCDE_S-R. The PCR product was digested with restriction enzymes *Not*I and *Sac*I and cloned in *Sac*I-*Not*I digested pBAD-ENSPA downstream the $P_{BAD}$ promoter (1). The resulted plasmid was named as pBAD-Csu. Deletions of genes of the CsuA/B, CsuA, CsuB, and CsuE subunit in pBAD-ABABCDE were constructed by reverse PCR using oligonucleotides Δ-CsuAB-R and -F, Δ-CsuA-R and -F, Δ-CsuB-R and -F and Δ-CsuE-R and -F, respectively. Replacements of residues 40-43 (LALA) to SGSG ($L_{40}ALA_{43}{\rightarrow}SGSG$), 140-145 (IVGIGV) to SSGSGS ($I_{140}VGIGV_{145}{\rightarrow}SSGSGS$), 157-159 (LGI) to SGS ($L_{157}GI_{159}{\rightarrow}SGS$) in CsuE were generated by reverse PCR and oligonucleotides CsuE-LALA_R and -_F, CsuE-IVGIGV_R and -_F and CsuE-LGI_R, respectively. The $L_{40}ALA_{43}{\rightarrow}SG$ mutation was introduced by accident, as a side product of the PCR intended to introduce the $L_{40}ALA_{43}{\rightarrow}SGSG$ mutation.

To express isolated N-terminal domain of CsuE (CsuE$_{NTD}$), synthetic nucleotide sequence encoding the first 205 residues of CsuE including the secretion sequence peptide was ordered from GenScript and inserted downstream of the *T7* promoter in the pET101D expression vector (Invitrogen). To facilitate the purification of the domain a C-terminal His tag was added to the expression plasmid using reverted PCR and primers CsuEN6Hrev and CsuEN6Hfwd. To improve the protein stability and increase the level of expression, codons for Pro206 and Asp207 were introduced preceding the His tag coding region by reverse PCR using primers CsuE-N6H_PR and CsuE-N6H_DF. The resulting plasmid was termed pET101-CsuE-NPD6H.

To generate the $I_{140}VGIGV_{145}{\rightarrow}SSGSGS$ mutation in CsuE$_{NTD}$ and CsuE co-expressed with CsuC, plasmids pET101-CsuE-NPD6H and pET101-CsuC6H-CsuE, respectively, were amplified using oligonucleotides CsuE-IVGIGV_R and CsuE-IVGIGV_F. The PCR products were blunt-end ligated, resulting in expression plasmids pET101-CsuE-NPD6H-SSGSGS and pET101-CsuC6H-CsuE-SSGSGS.

**Analysis of CsuE binding to plastics.** Wild type and $I_{140}VGIGV_{145} \rightarrow SSGSGS$ mutant CsuE complexed with the CsuC chaperone were purified essentially as described in (2). To enable detection of the protein by measuring time-resolved fluorescence, purified complexes were labeled with $Eu^{+3}$-chelate. 65 μM complex was incubated with 194 μM Kajo615 $Eu^{+3}$-chelate (Kaivogen) in 88.6 mM bicarbonate buffer, pH 9.8 at 4°C for 18 h. The reaction was stopped by adding 20 μl 3.0 M Tris-HCl, pH 8.45. Labeled proteins were kept on ice until gel filtration on a Superdex75 10/300 GL column (GE Healthcare) equilibrated with 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl (buffer A). Eluted proteins were concentrated on a Vivaspin device with 5 kDa molecular weight cut off (GE Healthcare). Protein concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

To ensure the comparability of the wild type and mutant binding experiments, the two were measured simultaneously, side-by-side. Labeled complexes (60 μg/ml in buffer A) were first incubated on ice in a 2 ml polypropylene tube (Sarstedt) for 30 min to ensure that the concentration is no longer changed due to binding to the plastic tube. 1 ml of the wild type and mutant proteins were pipetted simultaneously onto two 5.5 cm Petri dishes (Sarstedt) and shaken horizontally by hand to ensure the volume covered the entire bottom surface of the dishes. The binding experiment was done at 22°C with 250 rpm shaking on an automatic shaker (New Brunswick Scientific) to maintain uniformity of the sample volume. 7.2 μl samples were withdrawn at different time points and added into wells of a SpectraPlate$^{TM}$-96HB (PerkinElmer) plate for measuring $Eu^{+3}$ fluorescence. To estimate the initial concentration of the complex (0 time point of the reaction), 7.2 μl of the protein was taken for the fluorescence measurement directly from the tube. The samples were mixed for 10 to 15 min with 140 μl of Europium Fluorescence Intensifier (Kaivogen). As the fluorescence signal was very high, each sample was divided after mixing into three wells and the sum of signals from the three wells was used as the signal value. The delayed fluorescence was measured using a Victor3 1420 multi-label counter (PerkinElmer). The binding curves were corrected for evaporation from the plate. The evaporation effect was estimated by measuring the change in fluorescence of an unreactive form of the Kajo615 label incubated on a Petri dish in 1 ml buffer A, containing 1.94 pM BSA.

**Fig. S1.**
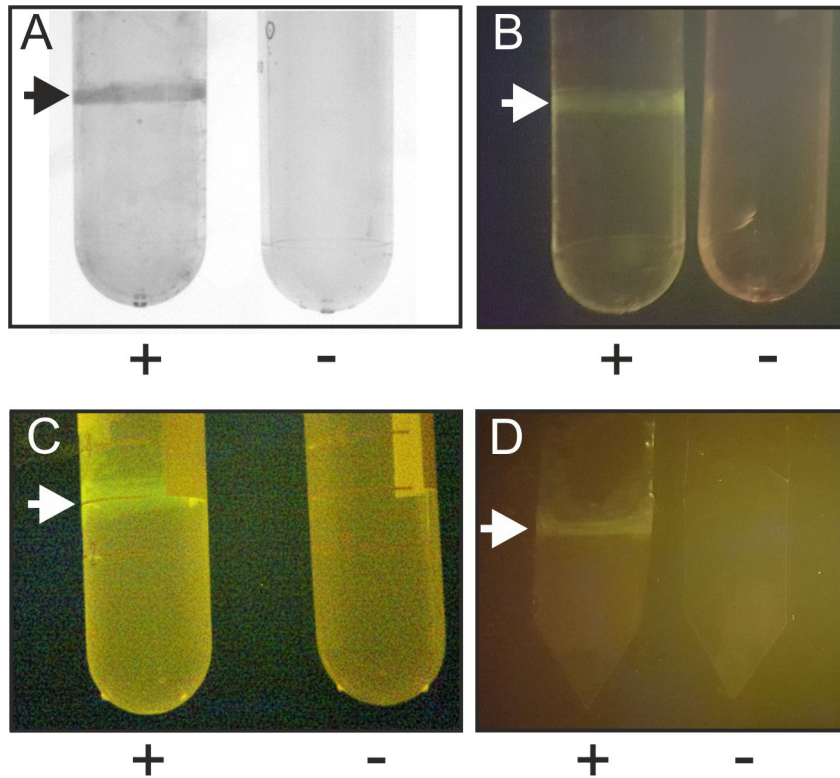


**Csu pili mediate biofilm formation of recombinant *Escherichia coli* on abiotic surfaces.** Csu pili expression was induced (+) or not induced (-) in *E. coli* harboring pBAD-Csu and pYFP plasmids. Bacterial cells were incubated in polystyrene (**A** and **B**) and polypropylene (**C**) tubes for 6 h at 37$^{\circ}$C in LB medium. Attached cells were detected by staining with crystal violet (**A**) or by observation of fluorescence owing to the expression of yellow fluorescence protein (**B** and **C**). To study binding to the hydrophilic surface of cellophane (**D**), straps of cellophane were added to the tubes and incubated with bacteria expressing or not expressing Csu pili, gently washed and analyzed as in (**B**) or (**C**). Although biofilm was observed on hydrophilic cellophane (**D**), cells were detached from these surfaces much more easily than from hydrophobic polystyrene, polypropylene or polyethylene surfaces.

**Fig. S2**
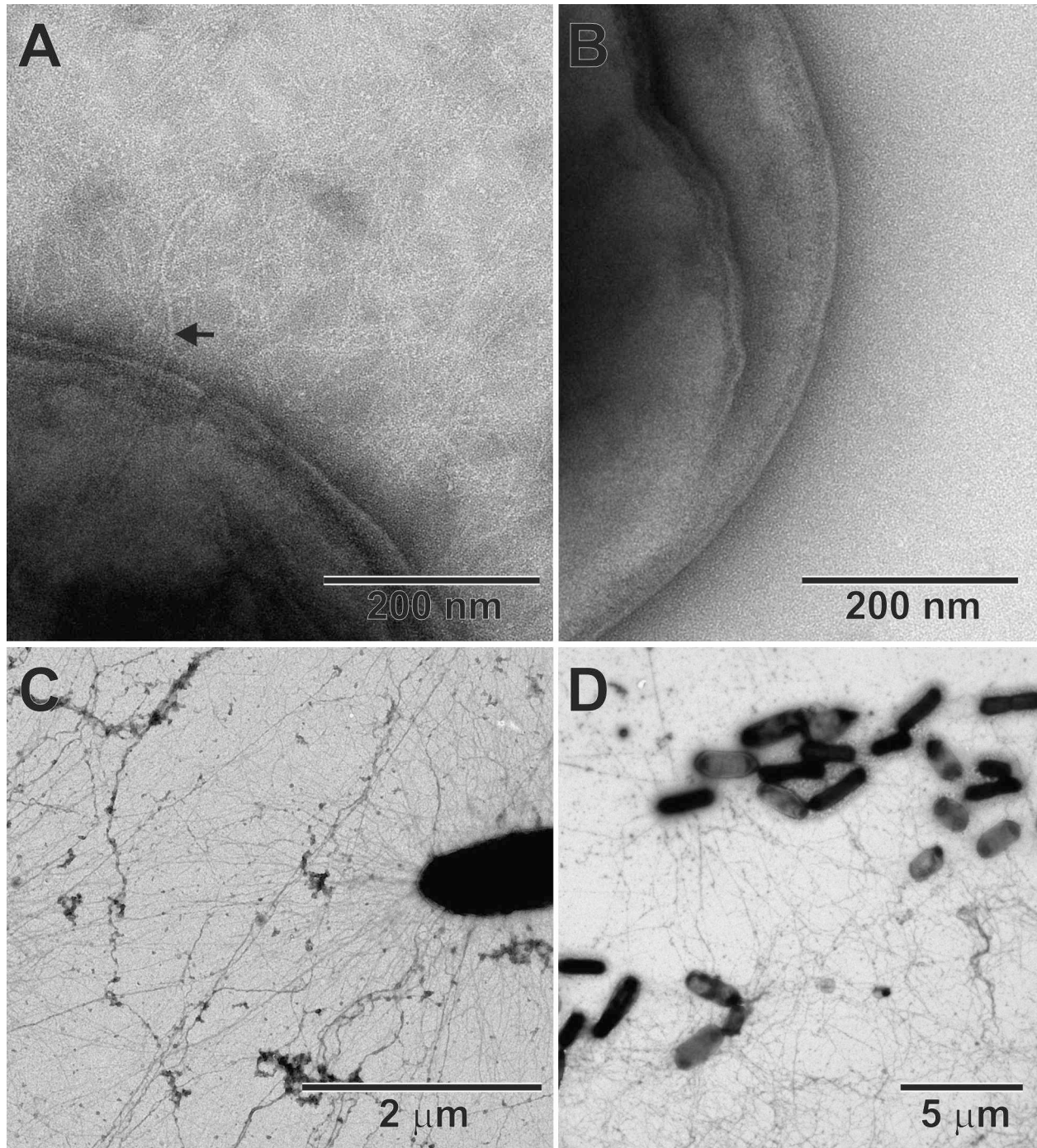


**Representative transmission electron microscopy micrographs of *E. coli* expressing Csu pili.** *E. coli* strain BL21 harboring the pBAD-ABABCDE plasmid was cultured in LB medium in presence (**A, C** and **D**) or absence (**B**) of arabinose and stained with ammonium molybdate. Micrographs of different magnification are shown. The arrow in **A** indicates an individual pilus clearly seen on the image. The Csu pilus is about 3.5 nm in diameter and is typically 1-2 μm long. However, some pili can reach 5 μm in length (3-6 times longer than the prototypical classical type 1 and P pili) and form long bundles well visible in (**D**).

**Fig. S3**
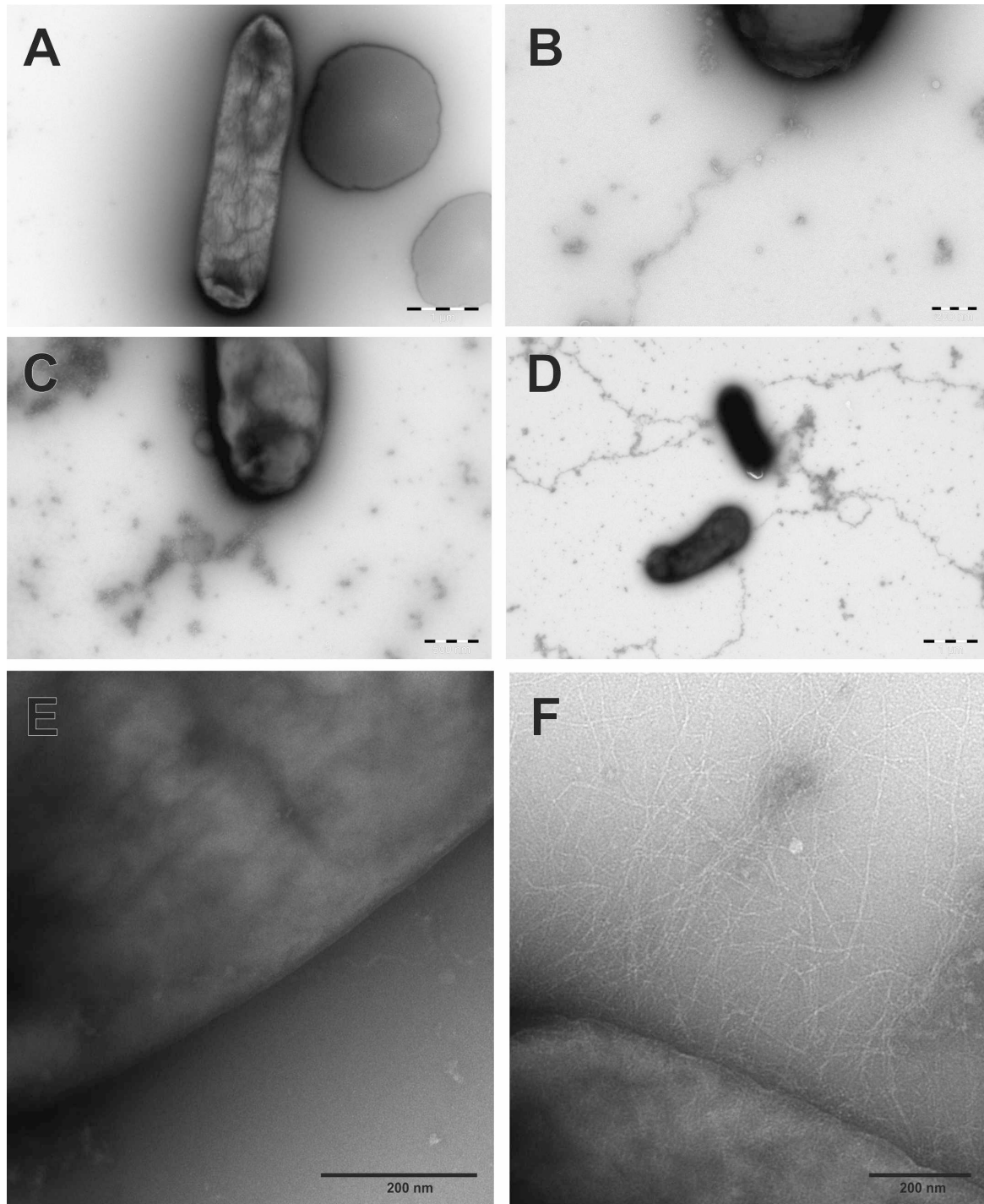


**Effect of mutations on Csu pilus expression.** *E. coli* harboring pBAD-ABABCDE derivatives, carrying deletions of entire genes of CsuA/B (**A**), CsuA (**B**), CsuB (**C** and **D**), CsuE (**E**) or a substitution of residues 140-145 in CsuE with SSGSGS ($I_{140}$VGIGV$_{145}$→SSGSGS, **F**), were cultured in LB medium in the presence of arabinose and stained with ammonium molybdate. Deletion of genes coding for CsuA/B and CsuE completely abolished the pilus assembly. ΔCsuA and ΔCsuB mutants expressed a few thick and long pili (**B-D**). The $I_{140}$VGIGV$_{145}$→SSGSGS mutation did not affect pilus assembly (**F**).

**Fig. S4**
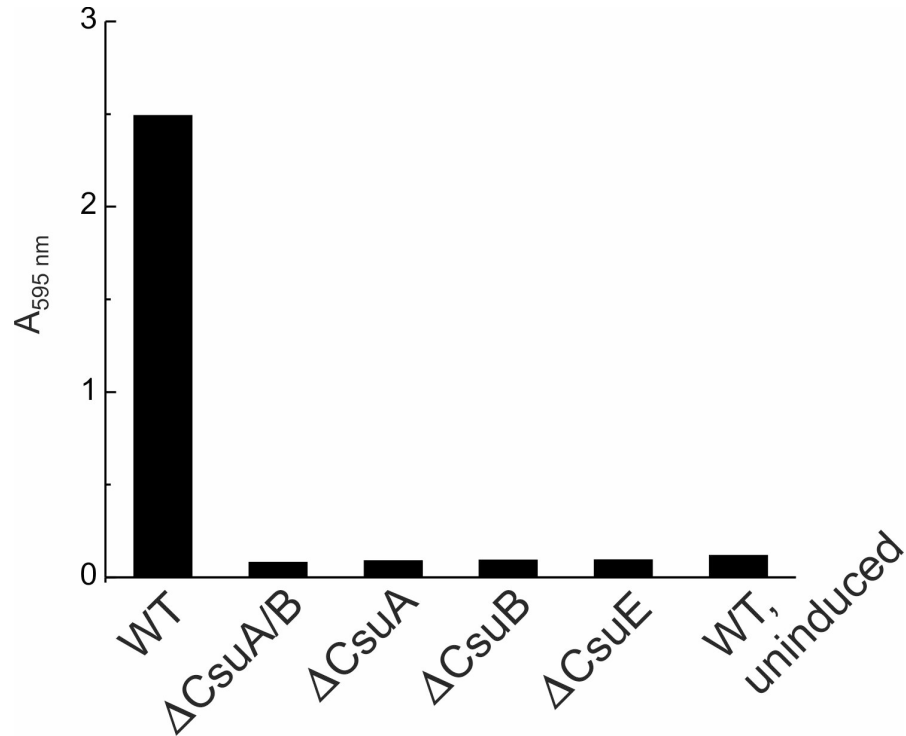


**Quantification of biofilms of *E. coli* harboring the wild type and subunit deleted *Csu* gene cluster.** An entire gene of CsuA/B, CsuA, CsuB or CsuE subunit was deleted (Δ) from the *Csu* gene cluster. Arabinose was added to induce pilus expression. Uninduced wild type (WT) cells were used as a negative control. Biofilms were stained with crystal violet, processed as described in Materials and Methods, and quantified by measuring absorbance at 595 nm.

**Fig. S5**
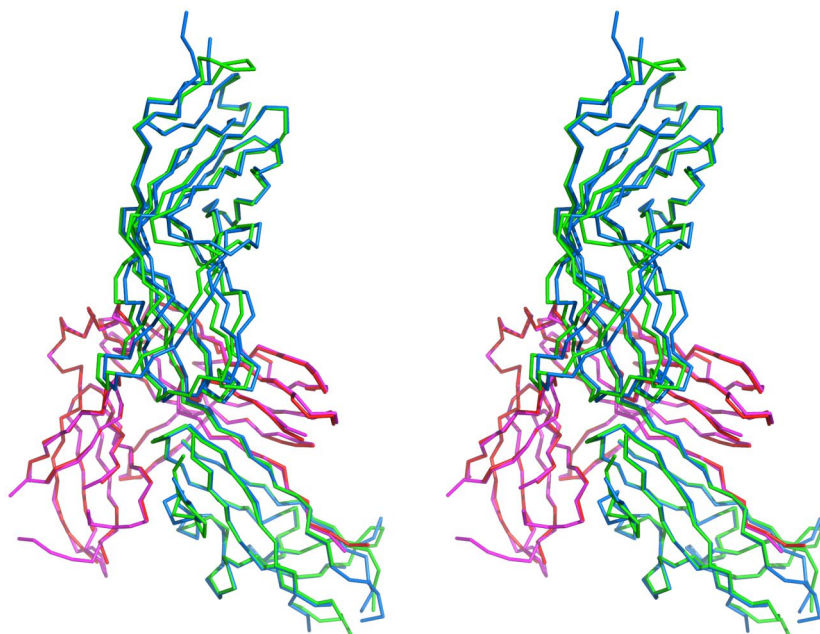


**Structural comparison of two copies of CsuC-CsuE in the asymmetric unit**. Molecules were superimposed by distance minimization between Cα atoms of CsuC. CsuC and CsuE are colored red and green in one complex and magenta and marine in another complex, respectively. Note that major differences are observed in the region of the N-terminal domain of CsuE. Most of these differences are caused by a change in the angle between domains in CsuE.

**Fig. S6**
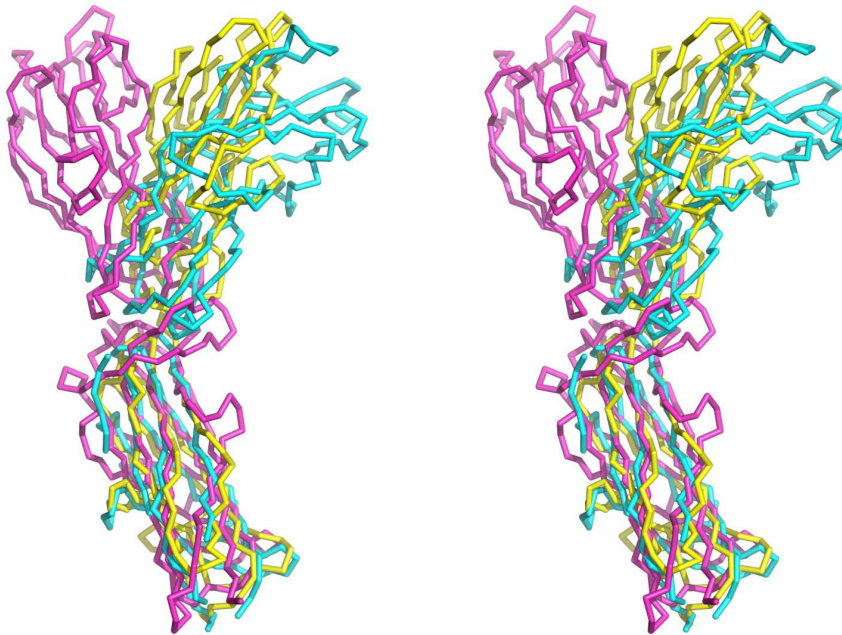


**The angle between N- and C-terminal domains in CsuE is sharper than that in two-domain adhesin subunits from the classical (FimH) and alternative (CfaE) CU systems.** CsuE (cyan), FimH (yellow), and CfaE (magenta) were superimposed by minimizing distances between Cα atoms in β-strands of their C-terminal, adaptor (pilin) domains. Stereo pairs of Cα-traces are shown. The angles between domains in CsuE, FimH, and CfaE are ~135°, 150°, and 180°, respectively, as measured from the center of gravity of each domain to that of the connector. The shape of CsuE is closer to that of the FimH adhesin from classical type 1 pili. The angle between domains in FimH (~150°) has been suggested to be a part of mechanism for shear-force-enhanced attachment to receptors (5).

**Fig. S7**



**A**

CsuE C-terminal domain
CsuE N-terminal domain

**B**

CsuE C-terminal domain
CsuA/B
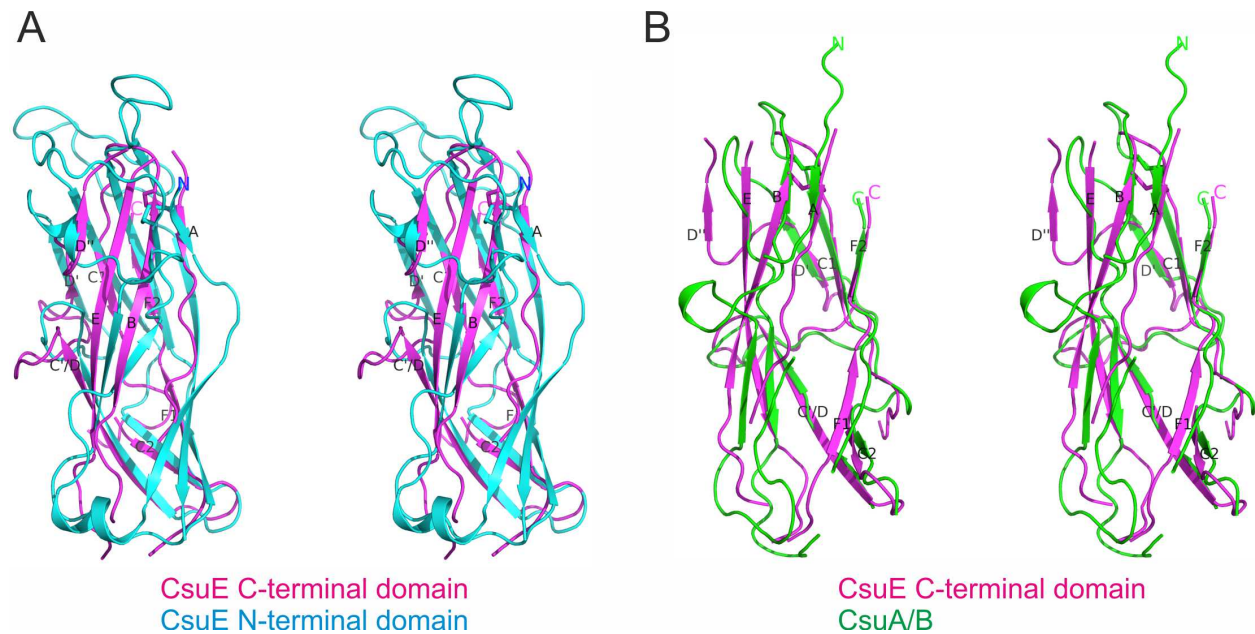
**C**

| Z-score; RMSD (Å); superimposed residues/total residues* | | | |
|---|---|---|---|
| | CsuE C-domain | CsuE N-domain | CsuA/B |
| CsuE C-domain | | 5.2; 4.0; 99/118 | 9.9; 2.6; 106/118 |
| CsuE N-domain | 5.3; 4.2; 101/179 | | 5.9; 3.8; 102/179 |
| CsuA/B | 9.9; 2.5; 106/130 | 5.9; 3.8; 102/130 | |

*Total residues of protein shown in the column

**Structural comparison of C- and N-terminal domains of CsuE between each other and the major CsuA/B pilus subunit of Csu pili.** (**A** and **B**) Cartoon diagrams of superpositions (stereo views) of C- (magenta) and N-terminal (cyan) domains of CsuE (**A**) and C-terminal domain of CsuE (magenta) and CsuA/B (green) (**B**). Disulfide bonds are shown as stick models. Secondary structure elements in the C-terminal domain of CsuE are labeled. CsuEpd and CsuA/B have a very different structure of β-sheet 1, ABED (**B**). In CsuA/B, β-sheet 1 is split in the middle due to the loop insertions that interrupt its β-strands as in most CU subunits (6). In contrast, β-sheet 1 in CsuEpd is uninterrupted, making the structure of CsuEpd more similar to the canonical Ig-like fold of the 8-stranded h-type (7). Consistent with the h-type fold, strand D switches sheets, but then repeats this twice. $CsuE_{NTD}$ and CsuEpd share a highly conserved disulfide bond connecting strands A and B (Fig. S11), but the structures of these domains differ substantially, and $CsuE_{NTD}$ is structurally distant from major pilin CsuA/B. (**C**) Structural comparison statistics. Superpositions and statistics were generated with the DaliLite server (http://ekhidna.biocenter.helsinki.fi/dali_lite/start) (8).

**Fig. S8**

A



B



C
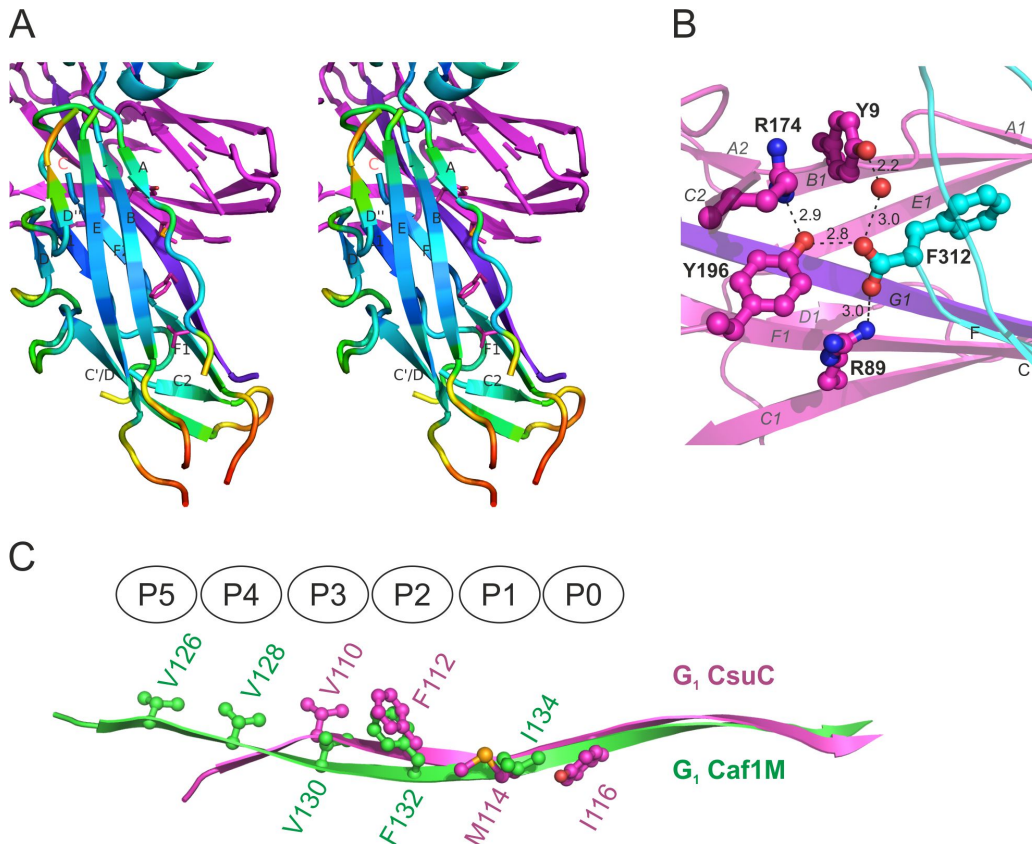


**Structure of the CsuC-CsuE complex highlights the characteristic features of non-classical assembly.** (**A**) Chaperone-bound pilin domain (CsuEpd) has a large fraction of disordered or poorly ordered sequence. Close-up of the CsuC-CsuE structure, showing the structure of CsuEpd (cartoon diagram, stereo view). CsuE is colored by B-factor of $C\alpha$ atoms with the color ranging from blue to red and corresponds to a B-factor range from 20 to 135 $Å^2$. CsuC is shown in magenta except for β-strand G1, which is violet. Donor strand residues are shown as sticks. Note the missing loops and elevated B-factors in the structure of CsuEpd distant from CsuC. (**B**) CsuC uses both domains to anchor the C-terminal carboxylate of CsuE rather than one as in the classical chaperones. Close-up of the CsuC-CsuA/B structure, demonstrating interactions between C-terminal carboxylate of CsuE and CsuC (cartoon diagram). The complex is painted as in Fig. 2. C-terminal Phe312 in CsuE and Tyr9, Arg89, Tyr196, and Arg174 in CsuC are shown as ball-and-stick. Hydrogen bonds are shown with dashes and their length is indicated. Residues Tyr9 and Arg89 belong to domain 1, whereas residues Tyr196 and Arg174 belong to domain 2. Tyr9 binds to the carboxylate via a structured water molecule (red sphere). This previously unidentified contact is also present in the CsuC-CsuA/B complex (6). (**C**) The donor strand motif in CsuC in the CsuC-CsuE complex is shifted towards the end of the $G_1$ strand relative to that in classical chaperones. Fragment of the superposition of CsuC-CsuE and Caf1M-Caf1 complexes, showing the alignment of $G_1$ donor strands in CsuC and Caf1M. Hydrophobic donor residues are shown as ball-and-stick and labeled. Positions of hydrophobic pockets P0-P5 in subunit acceptor clefts (P0-P3 in CsuE and P1-P5 in Caf1) are shown schematically above the donor strands.

**Fig. S9**
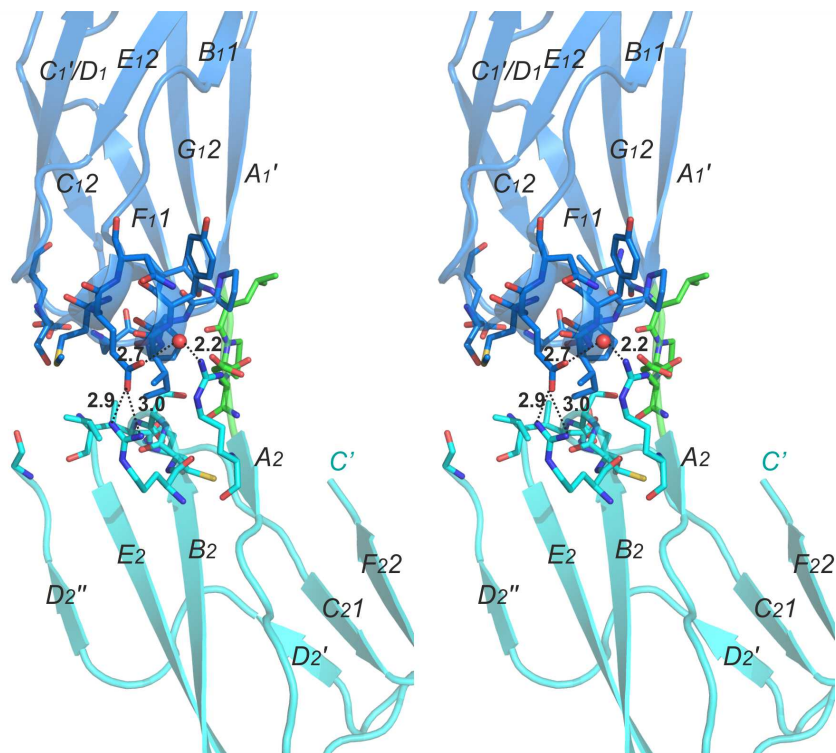


**Cartoon diagram of CsuE illustrating the interface between N- and C-terminal domains (stereo view).** N- and C-terminal domains are painted in blue and cyan, respectively. Residues engaged in interdomain contacts are shown as sticks. A structured water molecule participating in a network of hydrogen bonds between the two domains is shown as a red sphere. Hydrogen and ionic bonds are shown as dashed lines; the bond length is indicated. β-Strands and C-terminus are labeled. The buried area in Csu (383 Å$^2$) is only half of that in the CfaE subunit of CFA/I pili assembled via the alternative CU pathway, despite a similar size of the linker (9). This is explained by the striking difference in the angle between domains in these molecules (Fig. S6).

**Fig. S10**

A



B



C



CsuE          CfaE          FimH
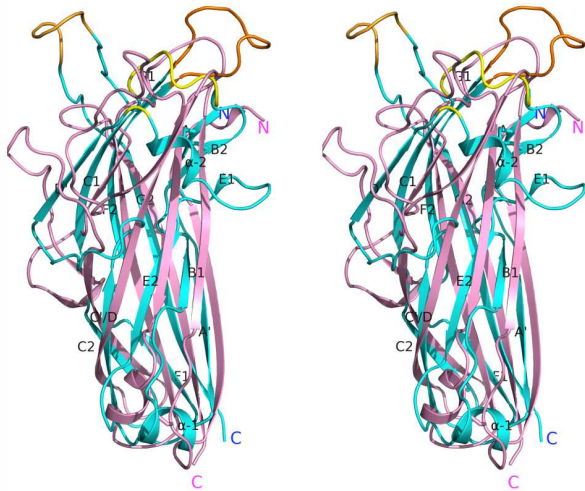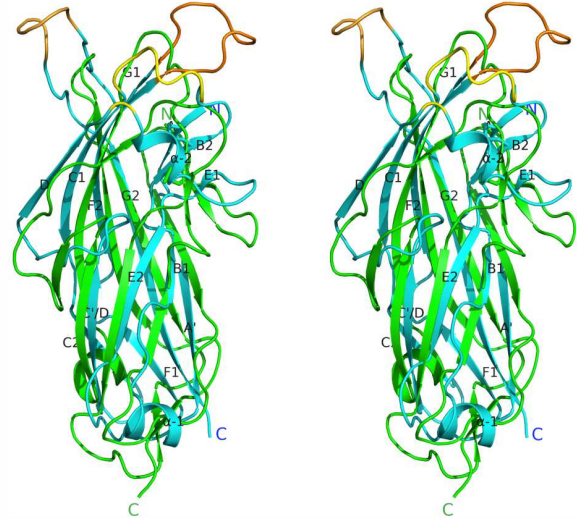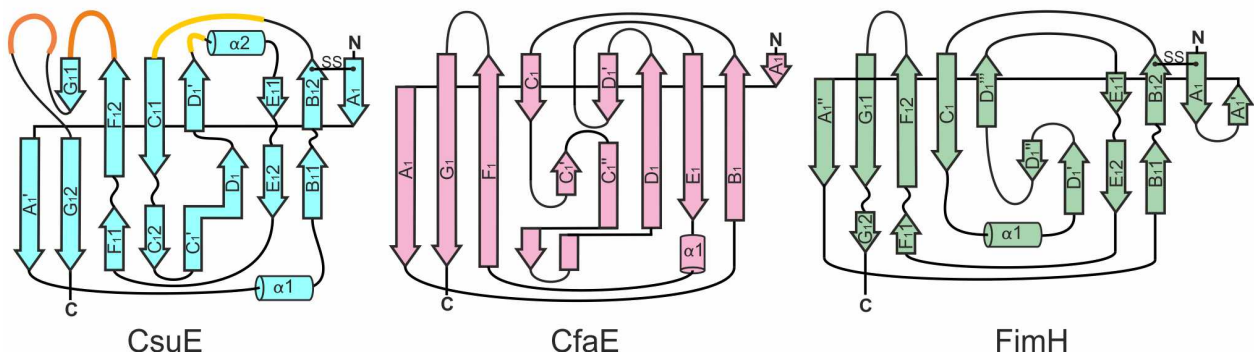
D

| Z-score; RMSD (Å); superimposed residues/total residues* | | | |
|---|---|---|---|
| | CsuE N-domain | CfaE N-domain | FimH N-domain |
| CsuE N-domain | | 6.9; 3.2; 114/179 | 5.5; 3.7; 116/179 |
| CfaE N-domain | 6.9; 3.2; 114/178 | | 4.5; 3.4; 98/178 |
| FimH N-domain | 5.5; 3.7; 116/158 | 4.5; 3.3; 98/158 | |

*Total residues of protein shown in the column

**Structural comparison of N-terminal domains of CsuE, CfaE, and FimH.** (**A** and **B**) Cartoon diagrams of superpositions (stereo views) of N-terminal domains of CsuE (cyan) and CfaE (pink) (**A**) and CsuE (cyan) and FimH (green) (**B**). Binding fingers 1-3 in CsuE are painted in yellow, orange, and light orange, respectively. Secondary structure elements in CsuE are labeled. Conserved disulfide bonds are shown as stick models. (**C**) Topology diagrams of CsuE, CfaE, and FimH. Strands and helices are shown as arrows and cylinders, respectively. (**D**) Structural comparison statistics.

# Fig. S11

Secondary structure labels (top): F₁2, G₁1, G₁2, A₂, B₂, C₂1

Sequence identifiers (upper block):

PAM18_4750/1-291
Q7S_18315/1-296
KOX_23800/1-297
SOD_c19210/1-288
YP_1720/1-292
PP1Y_Mpl9692/1-301
Entas_2592/1-302
ETA_04540/1-307
PP4_34580/1-300
YE1856/1-302
Pput_3332/1-301
PSEEN3394/1-301
H045_11630/1-304
M634_16975/1-302
PFL_3949/1-304
PAGR_g0744/1-305
EbC_38210/1-306
ESA_01347/1-298
ROD_19341/1-301
ECENHK_13825/1-302
Acav_4532/1-291
BMASAVP1_A1401/1-294
BPC006_I1682/1-294
MDS_2712/1-298
YPC_2125/1-292
SMWW4_v1c21200/1-288
ECA3077/1-297
NCGM2_0934/1-291
CsuE/1-312
BJAB0868_02454/1-312
A3UG_03070/1-299
P795_6510/1-297
PSYCG_09745/1-300
XCC1378/1-316
XAC1424/1-316
VAPA_1c20560/1-308
Sinme_6943/1-295
Bcep18194_A5756/1-299
Mesop_1922/1-295
BBK_506/1-296
Rta_34890/1-282
BYI23_D012110/1-291
ABO_0699/1-294
BRPE64_DCDS03180/1-302
AXYL_05495/1-300
Smlt1510/1-323
OA238_c46250/1-288
EntcI_2278/1-301
XOO1979/1-316

Secondary structure labels (lower block): C₂2, C₂'/D₂, D₂', D₂'', E₂, F₂1, F₂2

15

**Alignment of two-domain tip subunits from archaic chaperone/usher (CU) systems.**
Sequences of two-domain subunits from archaic CU gene clusters were identified in various genomes (see the table bellow) and analyzed with the *SignalP 4.1* server to detect and exclude secretion signal peptides. The predicted mature sequences were aligned with the program Clustal Omega at the EMBL-EBI website (10). The aligned sequences were edited and annotated using the program JalView (11). Conserved residues are color-coded according to their chemical properties: blue — hydrophobic, green — hydrophilic neutral, red — basic, magenta — acidic. Conserved cysteine residues are colored in pink. Glycine residues are shaded in orange, proline in yellow, aromatic residues in cyan. The sequences of two-domain subunits fall into families: the first family occupies the top part of the alignment table, above *A. baumannii* CsuE, (names of these sequences are shown in green), and the second family occupies the bottom part of the alignment table, below *A. baumannii* BJAB0868_02454 (names of these sequences are shown in blue). Completely invariant residues are indicated with an asterisk (*) in red and conserved positions containing less than 4% variable residues with an underlined asterisk (*) in red. Highly conserved regions (up to 15% variable residues) are marked with double dots (:) in red. Residues essential for semi-conservation (up to 25% non-matching residues) are dotted (.) in red. Conserved residues within each of the two families are indicated in a similar way, but the corresponding symbols are shown in green and blue for family 1 and 2, respectively. Secondary structure of the *A. baumannii* strain *19606* CsuE subunit (this paper) is shown above the alignment. Blue arrows and bars denote β strands and α helices, respectively.

## Sequence source

| # | Subunit name | Operon | Usher Accession | Chaperone Accession | Subunits Accession | Organism | Organism Class | Associated Disease | Operon DNA Accession |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PAM18_4750 | PAM18_4745-4750 | AEO77229.1 | AEO77228.1 | AEO77230.1 AEO77227.1 AEO77226.1 AEO77225.1 | *Pseudomonas aeruginosa M18* | *γ Proteobacteria* | Isolated from sweet melon rhizosphere | CP002496 |
| 2 | Q7S_18315 | Q7S_18290-18315 | AFE59873.1 | AFE59872.1 | AFE59874.1 AFE59871.1 AFE59870.1 AFE59869.1 | *Rahnella aquatilis HX2* | *γ Proteobacteria* | Isolated from a vineyard soil | CP003403 |
| 3 | KOX_23800 | KOX_23780-23800 | AEX06475.1 | AEX06474.1 | AEX06476.1 AEX06473.1 AEX06472.1 | *Klebsiella oxytoca KCTC 1686* | *γ Proteobacteria* | Bacterium is used in production of 2,3-butylene glycol and 2,3-butanediol from xylose | CP003218 |
| 4 | SOD_c19210 | SOD_c19210-c19260 | AGO54899.1 | AGO54900.1 | AGO54901.1 AGO54902.1 AGO54903.1 AGO54898.1 | *Serratia plymuthica 4Rx13* | *γ Proteobacteria* | A plant-associated, plant beneficial bacterium | CP006250 |
| 5 | YP_1720 | YP_1720-1725 | AAS61949.1 | AAS61950.1 | AAS61951.1 AAS61952.1 AAS61953.1 AAS61948.1 | *Yersinia pestis biovar Microtus str. 91001* | *γ Proteobacteria* | Causative agent of bubonic and pneumonic plague | AE017042 |
| 6 | PP1Y_Mpl9692 | PP1Y_Mpl9658-Mpl9692 | CCA90809.1 | CCA90808.1 | CCA90807.1 CCA90806.1 CCA90810.1 | *Novosphingobium sp. PP1Y Mpl megaplasmid* | *α Proteobacteria* | Isolated from a surface seawater sample collected from a closed bay | FR856861 |
| 7 | Entas_2592 | Entas_2592-2596 | AEN65322.1 | AEN65323.1 | AEN65324.1 AEN65325.1 AEN65321.1 | *Enterobacter asburiae LF7a* | *γ Proteobacteria* | An opportunistic pathogen | CP003026 |
| 8 | ETA_04540 | ETA_04540-04580 | CAO95501.1 | CAO95502.1 | CAO95503.1 CAO95504.1 CAO95500.1 | *Erwinia tasmaniensis strain ET1/99* | *γ Proteobacteria* | Non-phytopathogenic strain. Isolated from apple flowers. | CU468135 |
| 9 | PP4_34580 | PP4_34580-34640 | BAN55312.1 | BAN55313.1 | BAN55314.1 BAN55315.1 BAN55316.1 | *Pseudomonas putida NBRC 14164 DNA* | *γ Proteobacteria* | Saprotrophytic soil bacterium | AP013070 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | BAN55317.1 BAN55311.1 | | | | |
| 10 | *YE1856* | *YE1856-1861* | CAL11936.1 | CAL11937.1 | CAL11938.1 CAL11939.1 CAL11940.1 CAL11935.1 | *Yersinia enterocolitica subsp. enterocolitica 8081* | *γ Proteobacteria* | Causes infections in humans that usually lead to mild self-limiting enterocolitis or terminal ileitis and adenitis | AM286415 |
| 11 | *Pput_3332* | *Pput_3332-3338* | ABQ79459.1 | ABQ79460.1 | ABQ79461.1 ABQ79462.1 ABQ79463.1 ABQ79464.1 ABQ79458.1 | *Pseudomonas putida F1* | *γ Proteobacteria* | A versatile environmental isolate that is capable of growth on several aromatic hydrocarbons | CP000712 |
| 12 | *PSEEN3394* | *PSEEN3394-3400* | CAK16145.1 | CAK16146.1 | CAK16147.1 CAK16148.1 CAK16149.1 CAK16150.1 CAK16144.1 | *Pseudomonas entomophila str. L48 chromosome* | *γ Proteobacteria* | Entomopathogenic bacterium which, upon ingestion, kills Drosophila melanogaster | CT573326 |
| 13 | *H045_11630* | *H045_11630-11660* | AGE26392.1 | AGE26393.1 | AGE26394.1 AGE26395.1 AGE26396.1 AGE26397.1 AGE26391.1 | *Pseudomonas poae RE*1-1-14* | *γ Proteobacteria* | The endophytic bacterium shows broad antagonistic activity and is applied to seeds as a biocontrol agent to suppress late root rot in the sugar beet | CP004045 |
| 14 | *M634_16975* | *M634_16975-16995* | AGQ93894.1 | AGQ92617.1 | AGQ92618.1 AGQ92619.1 AGQ92616.1 | *Vibrio parahaemolyticus O1:Kuk str. FDA_R31 chromosome II* | *γ Proteobacteria* | A pathogenic marine bacterium that is the main causative agent of bacterial seafood-borne gastroenteritis in the United States | CP006005 |
| 15 | *PFL_3949* | *PFL_3955-3949* | AAY93214.1 | AAY93215.1 | AAY93213.1 AAY93216.1 AAY93217.1 AAY93218.1 AAY93219.2 | *Pseudomonas fluorescens Pf-5* | *γ Proteobacteria* | Pathogenic bacterium, usually affects patients with compromised immune systems | CP000076 |
| 16 | *PAGR_g0744* | *PAGR_g0744-g0749* | AER31282.1 | AER31283.1 | AER31284.1 AER31285.1 AER31280.1 | *Pantoea ananatis PA13* | *γ Proteobacteria* | Plant pathogen. Isolated from a diseased rice grain. | CP003085 |
| 17 | *EbC_38210* | *EbC_38170-38210* | CAX61351.1 | CAX61350.1 | CAX61352.1 CAX61349.1 CAX61348.1 | *Erwinia billingiae strain Eb661* | *γ Proteobacteria* | An epiphytic bacteria and may represent antagonists for biocontrol of fire blight | FP236843 |
| 18 | *ESA_01347* | *ESA_01343-01347* | ABU76606.1 | ABU76605.1 | ABU76607.1 ABU76604.1 ABU76603.1 | *Cronobacter sakazakii ATCC BAA-894* | *γ Proteobacteria* | Human pathogen. Enterobacter infection. | CP000783 |
| 19 | *ROD_19341* | *ROD_19341-19381* | CBG88687.1 | CBG88688.1 | CBG88689.1 CBG88690.1 CBG88686.1 | *Citrobacter rodentium ICC168* | *γ Proteobacteria* | Cause enteric disease with a high rate of fatality in mice and other rodents | FN543502 |
| 20 | *ECENHK_13825* | *ECENHK_13825-13845* | AFP70615.1 | AFP70616.1 | AFP70614.1 AFP70618.1 | *Enterobacter cloacae subsp. cloacae ENHKU01* | *γ Proteobacteria* | Nosocomial pathogen | CP003737 |
| 21 | *Acav_4532* | *Acav_4528-4532* | ADX48414.1 | ADX48413.1 | ADX48411.1 ADX48412.1 ADX48415.1 | *Acidovorax avenae subsp. avenae ATCC 19860* | *β Proteobacteria* | Causes seedling blight and bacterial fruit blotch of cucurbits. Under favorable environment, it becomes devastating and may cause 100% loss of marketable fruit. | CP002521 |
| 22 | *BMASAVP1_A1401* | *BMASAVP1 A1395-A1401* | ABM52929.1 | ABM52936.1 | ABM52947.1 ABM50447.1 ABM50929.1 ABM50824.1 | *Burkholderia mallei SAVP1 chromosome I* | *β Proteobacteria* | A phenotypic avirulent strain | CP000526 |

17

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|---|---|---|---|---|---|---|---|---|---|
| 23 | *BPC006_I1168 2* | BPC006_I16 77-I1682 | AFR15557.1 | AFR15556.1 | AFR15558.1 AFR15555.1 AFR15554.1 AFR15553.1 | *Burkholderia pseudomallei BPC006 chromosome I* | β Proteobacteria | Isolated from a melioidosis patient | CP00378 |
| 24 | *MDS_2712* | MDS_2707-2712 | AEB58742.1 | AEB58741.1 | AEB58743.1 AEB58740.1 AEB58739.1 AEB58738.1 | *Pseudomonas mendocina NK-01* | γ Proteobacteria | Isolated from farmland soil | CP002620 |
| 25 | *YPC_2125* | YPC_2119-2125 | ADV98708.1 | ADV98707.1 | ADV98710.1 ADV98706.1 ADV98705.1 ADV98704.1 | *Yersinia pestis biovar Medievalis str. Harbin 35* | γ Proteobacteria | Causative agent of bubonic and pneumonic plague | CP001608 |
| 26 | *SMWW4_v1c2 1200* | SMWW4_v1c 21200-v1c21250 | AGE17922.1 | AGE17923.1 | AGE17924.1 AGE17925.1 AGE17926.1 AGE17921.1 | *Serratia marcescens WW4* | γ Proteobacteria | A biofilm-forming bacterium isolated from paper machine aggregates | CP003959 |
| 27 | *ECA3077* | ECA3073-3076 | CAG75975.1 | CAG75974.1 | CAG75972.1 CAG75973.1 CAG75976.1 | *Erwinia carotovora subsp. Atroseptica SCRI1043* | γ Proteobacteria | Plant pathogenic bacterium, the causative agent of soft rot and blackleg potato diseases | BX950851 |
| 28 | *NCGM2_0934* | NCGM2_093 4-0939 | BAK87811.1 | BAK87812.1 | BAK87813.1 BAK87814.1 BAK87815.1 BAK87810.1 | *Pseudomonas aeruginosa NCGM2.S1 DNA* | γ Proteobacteria | The multidrug-resistant strain that caused an outbreak of urinary tract infection | AP012280 |
| 29 | *CsuE* | Ab_Csu(A/B) ABCDE | AAP43039.1 | AAP43038.1 | AAP43035.1 AAP43036.1 AAP43037.1 AAP43040.1 | *Acinetobacter baumannii 19606* | γ Proteobacteria | Pneumonia, meningitis, septicemia, and urinary and respiratory tract infections in immuno-compromised individuals | AY24169 6 |
| 30 | *BJAB0868_02 454* | BJAB0868_0 2454-02459 | AGQ11004.1 | AGQ11005.1 | AGQ11006.1 AGQ11003.1 AGQ11008.1 | *Acinetobacter baumannii BJAB0868* | γ Proteobacteria | Clinical isolate | CP003849 |
| 31 | *A3UG_03070* | A3UG_0307 0-03085 | AFM58362.1 | AFM58363.1 | AFM58364.1 AFM58361.1 | *Enterobacter cloacae subsp. dissolvens SDM* | γ Proteobacteria | Isolated from soil samples | CP003678 |
| 32 | *P795_6510* | P795_6495-6510 | AHB91026.1 | AHB91025.1 | AHB91027.1 AHB91024.1 | *Acinetobacter baumannii ZW85-1* | γ Proteobacteria | Isolated from diarrheal patient feces | CP006768 |
| 33 | *PSYCG_09745* | PSYCG_097 30-09745 | AGP49448.1 | AGP49447.1 | AGP49449.1 AGP49446.1 | *Psychrobacter sp. G* | γ Proteobacteria | Nonpathogenic soil bacterium, isolated from King George Island, Antarctica | CP006265 |
| 34 | *XCC1378* | XCC1381-1376 | AAM40677.1 AAM40678.1 | AAM40674.1 | AAM40676.1 AAM40679.1 | *Xanthomonas campestris str. ATCC 33913* | γ Proteobacteria | Causes a variety of plant disease | NC_00390 2 |
| 35 | *XAC1424* | XAC1427-1423 | AAM36296.1 AAM36297.1 | AAM36294.1 | AAM36295.1 AAM36298.1 | *Xanthomonas axonopodis pv. citri str. 306* | γ Proteobacteria | Exclusively pathogenic to a large group of plants such as citrus trees, rice, cotton, beans, and grapes | NC_00391 9 |
| 36 | *VAPA_1c2056 0* | VAPA_1c205 60-1c20590 | AGU49161.1 | AGU49162.1 | AGU49163.1 AGU49160.1 | *Variovorax paradoxus B4 chromosome 1* | β Proteobacteria | Degrades the organic thiol compound mercaptosuccinate, which could be a promising precursor for novel polythioesters | CP003911 |
| 37 | *Sinme_6943* | Sinme_6940-6943 | AEG58265.1 | AEG58264.1 | AEG58266.1 AEG58263.1 | *Sinorhizobium meliloti AK83 plasmid pSINME02* | α Proteobacteria | Plant endosymbiont | CP002785 |
| 38 | *Bcep18194_A 5756* | Bcep18194_ A5756-A5759 | ABB09353.1 | ABB09352.1 | ABB09350.1 ABB09351.1 | *Burkholderia sp. 383 chromosome 1* | β Proteobacteria | Isolated from forest soil | CP000151 |
| 39 | *Mesop_1922* | Mesop_1919- | AEH86402.1 | AEH86401.1 | AEH86403.1 | *Mesorhizobium* | α Proteobacteria | Isolated from B. | CP002279 |

18

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|---|---|---|---|---|---|---|---|---|
| | | | | AEH86400.1 | *opportunistum WSM2075* | | pelecinus plants | |
| 40 | *BBK_506* | *BBK 506-509* | AGZ27361.1 | AGZ28430.1 | AGZ28097.1 AGZ29774.1 | *Burkholderia pseudomallei NCTC 13179 chromosome 1* | *β Proteobacteria* | Human pathogen. Melioidosis. | CP003976 |
| 41 | *Rta_34890* | *Rta 34850-34890* | AEG94601.1 | AEG94600.1 | AEG94602.1 AEG94599.1 | *Ramlibacter tataouinensis TTB310* | *β Proteobacteria* | Isolated in the Tatauoine meteorite in the desert of South Tunisia | CP000245 |
| 42 | *BYI23_D0121 10* | *BYI23 D012 120-D012150* | AET94722.1 | AET94723.1 | AET94721.1 AET94724.1 | *Burkholderia sp. YI23 plasmid byi 1p* | *β Proteobacteria* | Isolated from a golf course soil | CP003090 |
| 43 | *ABO_0699* | *ABO_0702-0699* | CAL16149.1 | CAL16148.1 | CAL16147.1 CAL16150.1 | *Alcanivorax borkumensis* | *γ Proteobacteria* | Nonpathogenic marine bacterium | AM28669 0 |
| 44 | *BRPE64_DCD S03180* | *BRPE64_DC DS03190-03210* | BAN27255.1 | BAN27256.1 | BAN27257.1 BAN27254.1 | *Burkholderia sp. RPE64 plasmid p1 DNA* | *β Proteobacteria* | Isolated from field-collected *Riptortus pedestris* | AP013061 |
| 45 | *AXYL_05495* | *AXYL 05492-05495* | ADP18794.1 | ADP18793.1 | ADP18792.1 ADP18795.1 | *Achromobacter xylosoxidans A8* | *β Proteobacteria* | Isolated from soil contaminated with polychlorinated biphenyls. | CP002287 |
| 46 | *Smlt1510* | *Smlt1508-1513* | CAQ45047.1 | CAQ45048.1 AQ45044.1 | CAQ45049.1 CAQ45046.1 | *Stenotrophomon as maltophilia K279a* | *γ Proteobacteria* | Human pathogen isolated from a patient undergoing chemotherapy developed a bloodstream infection that did not respond to therapy with piperacillin/tazobacta m, ceftazidime or imipenem | AM74316 9 |
| 47 | *OA238_c4625 0* | *OA238 c462 10-c46250* | AGI74478.1 | AGI74477.1 | AGI74479.1 AGI74475.1 | *Octadecabacter arcticus 238* | *α Proteobacteria* | Isolated from sea ice of the Arctic | CP003742 .1 |
| 48 | *Entcl_2278* | *Entcl 2275-2278* | ADO48529.1 | ADO48528.1 | ADO48527.1 ADO48530.1 | *Enterobacter lignolyticus SCF1* | *γ Proteobacteria* | Isolated anaerobically from tropical forest soils | CP002272 |
| 49 | *XOO1979* | *XOO1982-1978* | AAW75234.1 | AAW75232.1 AAW75235.1 | AAW75233.1 AAW75236.1 | *Xanthomonas oryzae pv. oryzae KACC10331* | *γ Proteobacteria* | Causes bacterial blight of rice | AE013598 |

**Fig. S12**

<span style="color:blue">MNIKTKKLLRHLCMFSGLMLTGNMAHA</span>A<u>C</u>SVSASGTSSISVPSIYLMENGENSSQFNSGLS<u>C</u>TGF
SLALANMTYLKYRVEQMSNSFTNAQTGEKLNAIILDSNNEIISLGQEKDMSSFTLVNLFSGPDGN
LPFYIRLPAGQSVSPGVYQADSPLKVKWFYSVPAVAIVGIGVFFESPGFRRGALGIGFNWGSGAD
SLGSLSITVLPD<span style="color:green">HHHHHH</span>

**Protein sequence of N-terminal domain of CsuE tagged with six histidine residues.** Signal peptide and His$_6$-tag are shown in blue and green, respectively.

**Fig. S13**
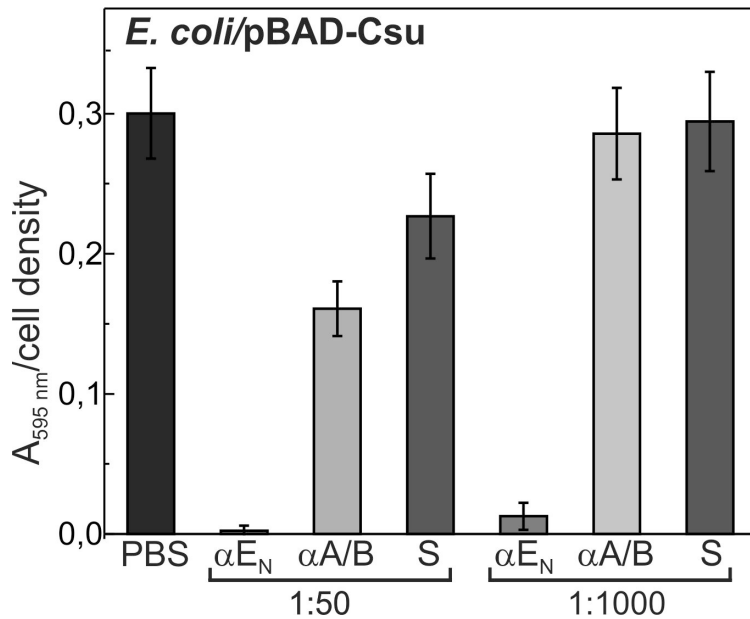


**Anti-CsuE antibody blocks Csu-pili mediated biofilm formation.** *E.* coli/pBAD-Csu cells overexpressing Csu pili were preincubated with PBS buffer, 1:50 and 1:1000 dilutions of antibodies raised against the N-terminal domain of CsuE ($\alpha$E$_N$) and CsuA/B ($\alpha$A/B) or the preimmune serum (S) and then assayed for biofilm formation in polystyrene microtiter plates. Biofilms were quantified by crystal violet staining and measuring absorbance at 595 nm. The results are representative of three independent experiments.

**Fig. S14**
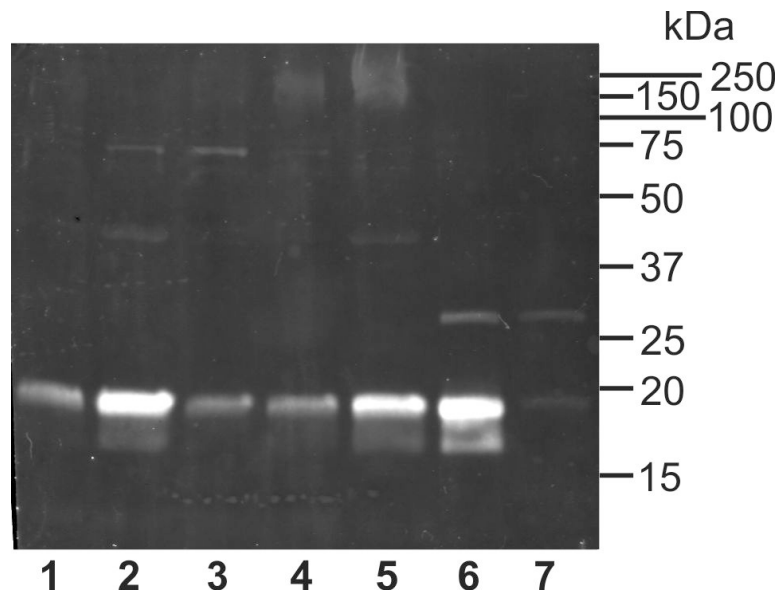


**Csu pili are constitutively expressed and assembled on the cell surface of different *Acinetobacter baumannii* strains**. Surface extracts prepared from *A. baumannii* strains 59798, 60611, 68164, 890, and 19069T from the Culture Collection of the University of Gothenburg (CCUG), lanes 1-5, respectively, and *E. coli* BL21 harboring pBAD-Csu in the presence (lane 6) and absence (lane 7) of arabinose, inducing Csu pili expression, were analyzed by Western blotting. The blot was probed with anti-CsuA/B rabbit serum followed by detection with IRDye 68RD-conjugated goat anti-rabbit antibody. The mobility of molecular mass markers is shown to the right in the figure.

**Table S1.**

**Strains**

| *Escherichia coli* | | | | | |
|---|---|---|---|---|---|
| BL21-AI | *E. coli* strain B F⁻ *ompT gal dcm lon hsdS$_B$($r_B^-$ $m_B^-$) [malB⁺]$_{K-12}$(λ$^S$) araB::T7RNAP-tetA* | | | | |
| *Acinetobacter baumannii* | | | | | |
| **Strain [a]** | **Source** | **Country** | **Year** | **Csu pili expression level [b]** | **Anti-CsuE serum dilution required for biofilm inhibition [c]** |
| CCUG 19096 T | Urine | France | 1986 | +++ | 1:1000 |
| CCUG 60611 | Blood | Netherlands | 1997 | +++ | 1:1000 |
| CCUG 68164 | Abdomen | Sweden | 2006 | ++ | 1:1000 |
| CCUG 59798 | Urine | Sweden | 2010 | + | 1:1000 |
| CCUG 890 | Urine | Great Britain | 1970 | + | 1:1000 |

[a] All strains were from the Culture Collection, University of Gothenburg (CCUG)
[b] Estimated from the intensity of the band of CsuA/B in Western blots (Fig. S14)
[c] Example of the experiment is shown in Fig. 4.

**Table S2.**

**Oligonucleotides**

| Name | Sequence (5'→3') |
|---|---|
| CsuABABCDE_N-F | AAGCGGCCGCAGATTAGCCATATTTTATTTGTCGAG |
| CsuABABCDE_S-R | TTGAGCTCTTAAAGATAAAAGCCCATGAACTGAG |
| Δ-CsuAB-R | GAATTCCGTTAATTCCTCCTGTTAG |
| Δ-CsuAB-F | GAGTAGCAGGTTTGCTCAAATATG |
| Δ-CsuA-R | TTAGAAATTTACAGTGACTAATAGAGTATC |
| Δ-CsuA-F | TAGTACCAATTACGATTGAGTTTTAAG |
| Δ-CsuB-R | TTTTCTTAAAACTCAATCGTAATTGGTAC |
| Δ-CsuB-F | ACGGCCGGAATTTATAAAGATACTG |
| Δ-CsuE-R | TCATGGCAAAGATACCTCGTGA |
| Δ-CsuE-F | TAAAAGCTGTTTTATATAGGAGATAAAAAG |
| CsuE-LALA_R | ACCCGAGCTAAAACCCGTACACGACAAAC |
| CsuE-LALA_F | TCAGGAAACATGACCTACCTAAAATATCGGGTTG |
| CsuE-IVGIGV_R | TCCCGAACTCGCGACTGCGGGCACAGAATAG |
| CsuE-IVGIGV_F | AGCGGGTCGTTCTTTGAGAGTCCTGGGTTTAG |
| CsuE-LGI_R | CCAATGCACCGCGTCTAAACCCAGG |
| CsuE-LGI_F | GTATTGGTTTTAACTGGGGAAGTGGGGC |
| CsuEN6Hrev | GTGATGATGAAGTACGGTAATTGAGAGTGAAC |
| CsuEN6Hfwd | CATCACCATTAATGAAAGGGCGAGCTC |
| CsuE-N6H_PR | TGGAAGTACGGTAATTGAGAGTGAAC |
| CsuE-N6H_DF | GACCATCATCACCATCACCATTAATG |

**Table S3**

**Refinement statistics**

| Data set | Native data |
|---|---|
| **Resolution** | 53.9-2.31 |
| **Number of reflections** | |
| - Total | 87930 |
| - Work set | 83189 |
| - Test set | 4741 |
| $R_{work}/R_{free}$ **(%)** | 21.18/25.87 |
| **Number of atoms** | |
| - Protein | 7688 |
| - Solvent | 178 |
| **Number of protein residues** | 1000 |
| **Number of modified residues** | 6 (N-dimethyllysine)[a] |
| **Number of ligands/ions** | 0 |
| **Wilson B-factor ($\text{Å}^2$)** | 54.01 |
| **Average B-factor ($\text{Å}^2$)** | |
| - Main chain | 65.77 |
| - Side chain | 78.15 |
| - Solvent | 55.20 |
| **Rmsd stereochemistry** | |
| - Bond lengths (Å) | 0.0043 |
| - Bond angles (°) | 1.050 |
| **Rmsd B-factors** | |
| - Main chain | 4.106 |
| - Side chain | 4.982 |
| **Ramachandran analysis[b]** | |
| Residues in outlier regions | 0.73% |
| Residues in favored regions | 95.7% |
| Residues in allowed regions | 99.27% |

[a] Methylated lysine residues
[b] Output from Molprobity (outliers motivated by electron density)

*SI* **References**

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